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CELLULAR MECHANISMS OF CENTRAL NERVOUS MODULATION

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Final Report  
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### Scientific activities

In a previous report, an account was given of a technique for selective disruption of cockroach neuroglia using ethidium bromide as a glial toxin. This compound (which binds to both DNA and RNA) disrupts the glial nuclei in central nervous connectives but does not affect axons (whose nuclei are contained in the ganglia). As described in the previous report, we have found that there is a rapid and ordered repair of the peripheral glial elements following selective disruption.

A consistent feature of the early stages of glial damage is the prominent involvement of granule-containing cells which were never seen in control preparations. It was suggested that the granule-containing cells, which share a number of cytological features with haemocytes play an integral part in glial repair.

More recently, we have carried out experiments designed to test the hypothesis that the granule-containing cells are, in fact, derived from haemocytes. We have three lines of evidence which support this hypothesis. First, in cultured cords (which can maintain axonal excitability for longer than a month) there is no appearance of granule-containing cells at the periphery of ethidium treated preparations. Secondly, in double-ligatured ethidium-treated connectives (in which there is no direct access to untreated portions of the nervous system) granule-containing cells still appear.

A third line of evidence is derived from experiments using fluorescent carboxylated microspheres (0.27 and 0.57 microns in diameter). When injected into the blood there is a rapid uptake of microspheres into a relatively small proportion (<10%) of the haemocytes (as revealed by combined Nomarski and U.V. epifluorescent microscopy).

Unexpectedly the injection and uptake of the inert microparticles grossly interrupted peripheral glial repair. Electron micrographs showed a virtual absence of granule-containing cells while electrophysiological

experiments indicated a greatly delayed re-establishment of the blood-brain barrier, as indicated by the access of potassium ions to the axon surfaces. This evidence lends support to the notion that the haemocytes are involved in glial repair and that this repair can be severely perturbed when the haemocytes are otherwise pre-occupied.

Only very occasionally could apparently repairing cells be seen at the periphery in ethidium-treated connectives, following injection and uptake of microspheres by the haemocytes. When they did some were found to contain microspheres - in electron micrographs. This again can be interpreted as evidence that the haemocytes are involved in peripheral glial repair.

We have also found that some cancer drugs have a profound effect on glial repair, following selective glial damage. One of the drugs that we have used is bleomycin, a compound which prevents cell proliferation by breaking up DNA.

At a dosage of 0.30 and 0.60  $\mu\text{g}$  per cockroach, bleomycin greatly delays neural repair following ethidium treatment. For example, even after 14 days the axons are still accessible to extraneously-applied potassium ions as judged by the rate of decline of the intracellularly-recorded action potentials on exposure to high-potassium saline. Electronmicrographs also show a paucity of peripheral granule-containing cells.

This effect of bleomycin suggests that the circulating haemocytes are not adequate to initiate repair and that the ones involved must be produced by cell proliferation induced by neural damage.

#### Publications

Papers listed in the two previous reports have been published, accepted for publication or are still in the process of being reviewed. A remaining publication is:

Lecch, C.A. (1984). Ethidium bromide effects on cells of the cockroach central nervous system. J. Cell Sci. (in the press).

#### Future Research Plans

Dr. P.K. Schofield is currently engaged in the final phase of the research on the effects of octopamine on modulation of the permeability and electrical properties of the perineurial glia.

In vivo studies on the effects of cancer drugs on glial repair will be continued. Comparing the effects of compounds which can cross the blood-brain barrier with those of water-soluble ones which are excluded. This should help resolve the role of endogenous cells which, for a variety of reasons, we believe to be involved in neural repair at a deeper level following selective glial disruption.

We propose to follow, in detail, repair of glia in cultured cords (in which there can be no haemocyte involvement) and, in particular, to follow thymidine labelling of neuroglia following lesioning and selective glial disruption.

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